Citation:

Rajaram S, Haddad EH, Mejia A, Sabaté J. Walnuts and fatty fish influence different serum lipid fractions in normal to mildly hyperlipidemic individuals: a randomized controlled study.*Am J Clin Nutr*. 2009 May;89(5):1657S-1663S.

PubMed ID: 19339404

Study Design:

Randomized Crossover Trial

Class:

A - Click here for explanation of classification scheme.

Research Design and Implementation Rating:

NEUTRAL: See Research Design and Implementation Criteria Checklist below.

Research Purpose:

To determine whether walnuts (plant n-3 fatty acid) and fatty fish (marine n-3 fatty acid) have similar effects on serum lipid markers at intakes recommended for primary prevention of CHD.

Inclusion Criteria:

- Normolipidemic or mildly hyperlipidemic but apparently healthy subjects

Exclusion Criteria:

- Consumption of nuts or fish more than twice a week
- Drink caffeinated beverages more than three times a day
- Drink alcohol more than twice a week
- Food allergies
- Smoke
- History of chronic or metabolic disease
- Regularly use medication or supplements known to affect blood lipids
- Women with irregular menses or started hormone treatment within the past five years
- Serum total cholesterol concentration more than 7.76 mmol/L and triglyceride concentration more than 3.33 mmol/L

Description of Study Protocol:

Recruitment : The subjects were recruited through advertisements in and around Loma Linda University.

Design: Randomized crossover trial

Blinding used (if applicable): implied with laboratory measures

Intervention (if applicable):

- The study had a three diet periods of four weeks each with a weekend break in-between diet periods.
    - Walnuts, six days per week: the diet was identical to the control diet except that the walnuts were substituted by meats and dairy products. The amount was based on the Food and Drug Administration qualified health claim for walnuts
    - Salmon, twice a week: the fish diet was identical to the control diet except that the cooked salmon substituted for the same amount of other types of meats twice a week. The dosage of fish was based on the recommendation of the American Heart Association
    - Control diet- excluded nuts and fatty fish
- Before beginning the dietary treatments, participants received and average American diet that contained 34% energy from fat for one week (run-in period).
- Subjects were randomized and stratified on the basis of age, gender, and baseline serum total cholesterol concentration to one to six possible diet sequences in a crossover fashion for four weeks each.
- All diets were designed to follow the current Dietary Guidelines for Americans.
- All meals were prepared in the university's metabolic kitchen, and Sunday through Friday of each week, all the subjects ate breakfast and dinner at this facility.
- Whereas daily lunch, snacks, and Saturday meals were packaged for consumption at the participant's discretion.
- Menus were designed for seven energy intake values, ranging from 1800-3600 kcal/day.
- Participants were weighed twice a week and their energy intake adjusted as needed to maintain a stable body weight.
- Quality control was insured by weighing foods before being served, with one investigator present at all meal times, and by requiring participants to maintain a daily diary to record food consumption.

Statistical Analysis:

- Mixed linear models with fixed terms for diet, treatment period, clinic day, period-by-clinic-day interaction, and a random term for subjects
- A significant main effect of diet was indicated when the P value of the F test for the main effect was <0.05
- When the main effect of the diet was significant, individual treatments were assessed using Turkey-Kramer method for adjusted multiple comparison
- A difference with $P<0.05$ was considered to be significant

Data Collection Summary:

Timing of Measurements: Biochemistry parameters were measured on two alternate study days at the end of each diet period, including baseline. The erythrocyte membrane fatty acid composition was measured at the end of each diet period.

Dependent Variables

- Erythrocyte membrane of fatty acid composition: saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), polyunsaturated fatty acid (PUFA), linoleic acid (LA),

alpha-linoleic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), used as a marker of compliance
- Total cholesterol concentration
- LDL cholesterol concentration
- HDL cholesterol concentration
- LDL cholesterol:HDL cholesterol
- Total cholesterol:HDL cholesterol
- Apolipoproteins (apo A-I and apo B)
- apo B:apo A-I
- LDL cholesterol:apoB
- Triglyceride

Independent Variables

- Walnut diet (42.5 g or 1.5 oz/2400 kcal)
- Fish diet/salmon (113g or 4 oz raw)
- Control diet

Control Variables

- Level of hyperlipidemia at baseline
- Age
- Physical activity

Description of Actual Data Sample:

Initial N: 27

Attrition (final N): 25 (14 males, 11 females). Reason for drop out: time conflict (2).

Age: ranged from 23 to 65 years old

Ethnicity: not reported

Other relevant demographics: The mean baseline total cholesterol was 5.41 mmol/L, mean baseline LDL cholesterol was 3.53 mmol/L, and mean baseline triglyceride was 1.25 mmol/L.

Anthropometrics: The mean BMI was 24.8 kg/m^2 (range: 18.7-36.6 kg/m^2). Mean body weight at the end of treatments were not different from each other: control diet and fish diet, mean was 71.7 kg; walnut diet, mean was 71.9 kg.

Location: Loma Linda, CA

Summary of Results:

Key Findings

- Walnut diet decreased total cholesterol (4.87$\pm$0.18 mmol/L) and LDL cholesterol (2.77$\pm$0.15 mmol/L) significantly (P<0.0001) when compared to fish diet (5.33$\pm$0.18 mmol/L; 3.20$\pm$0.15 mmol/L, respectively) and control diet (5.14$\pm$0.18mmol/L; 3.06$\pm$0.15 mmol/L, respectively);

- Fish diet resulted in significantly decrease serum triglyceride (1.00±0.11 mmol/L) and increase HDL-cholesterol concentrations (1.23±0.05 mmol/L) compared with control diet (1.12±0.11 mmol/L and 1.19±0.05 mmol/L, respectively; P<0.05), and walnut diet (1.11±0.11 mmol/L, P<0.05 and 1.18±0.05 mmol/L, respectively; P<0.001)
- The ratios of total cholesterol:HDL cholesterol (P=0.02), LDL cholesterol:HDL cholesterol (P<0.0005), and apo B:apo A-I (P<0.0001) all were significantly lower in those who followed the walnut diet compared with subjects who followed the control and fish diets

Other Findings

- The fish diet resulted in significantly higher serum total cholesterol (5.33±0.18 mmol/L) and LDL cholesterol (3.20±0.15 mmol/L) compared with those in the control (P<0.02) and walnut diets(P<0.0001). This effect was magnified in subjects with higher baseline values for these variables. Subjects with moderate to high baseline of total cholesterol (<5.17 mmol/L) and LDL cholesterol (>3.36 mmol/L) values, the increase in these variables was progressively greater in those who followed the fish diet.
- The fish and control diets had slightly higher amounts of cholesterol but still less than 350 mg/day. The percentage of energy from total PUFA, specifically linoleic acid and alpha linolenic acid (ALA) were higher in the walnut diet compared with the other two diets. However the fish diet had higher amounts of EPA and DHA compared with negligible amounts (<0.05 mg) in the other diets

Fatty acid composition of erythrocyte membranes at the end of each of the three treatment diets

Fatty acid	Control diet	Fish diet	Walnut diet	*P value
SFA	mol% 38.9±0.39^{a}	mol% 40.2±0.39^{b}	mol% 39.9±0.39 ^{a,b}	0.0409
MUFA	15.8±0.19^{a}	15.8±0.19^{a}	14.5±0.19^{b}	<0.0001
PUFA	38.6±0.38^{a}	39.4±0.38^{a}	41.0±0.38^{b}	<0.0001
LA (18:2n-6)	10.5±0.29^{a}	9.7±0.29^{a}	12.4±0.29^{b}	<0.0001
ALA (18:2n-3)	0.13±0.01^{a}	0.12±0.01^{a}	0.26±0.01^{b}	<0.0001
EPA(20:5n-3)	0.51±0.04^{a}	1.08±0.04^{b}	0.58±0.04^{a}	<0.0001
DHA (22:6n-3)	4.52±0.21^{a}	5.12±0.21^{b}	4.46±0.21^{a}	0.0002

*P values for the main effect of diet obtained from a mixed linear model. Values within a row with different superscript letters are significantly different, P<0.05.

Author Conclusion:

Our findings suggest that individuals who have mild to moderate hyperlipidemia and who need to lower cholesterol may consider adding 1.5 oz per day of walnuts to a heart healthy diet, whereas those who need to lower triglyceride may benefit from eating at least two servings of fatty fish per week.

Reviewer Comments:

- *Possibility of carry-over effect due to the short wash-out period between treatment, which jeopardize the treatment effect (outcome)*
- *Small sample increases risk of type II error*
- *Subjects were mainly healthy with only mild to moderate hyperlipidemia, therefore, results may be limited to this specific population*

Research Design and Implementation Criteria Checklist: Primary Research

Relevance Questions		
1.	Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (Not Applicable for some epidemiological studies)	Yes
2.	Did the authors study an outcome (dependent variable) or topic that the patients/clients/population group would care about?	Yes
3.	Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to nutrition or dietetics practice?	Yes
4.	Is the intervention or procedure feasible? (NA for some epidemiological studies)	Yes

Validity Questions		
1.	**Was the research question clearly stated?**	Yes
1.1.	Was (were) the specific intervention(s) or procedure(s) [independent variable(s)] identified?	Yes
1.2.	Was (were) the outcome(s) [dependent variable(s)] clearly indicated?	Yes
1.3.	Were the target population and setting specified?	Yes
2.	**Was the selection of study subjects/patients free from bias?**	No
2.1.	Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study?	No
2.2.	Were criteria applied equally to all study groups?	Yes
2.3.	Were health, demographics, and other characteristics of subjects described?	???
2.4.	Were the subjects/patients a representative sample of the relevant population?	No
3.	**Were study groups comparable?**	Yes

3.1.	Was the method of assigning subjects/patients to groups described and unbiased? (Method of randomization identified if RCT)	???
3.2.	Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline?	Yes
3.3.	Were concurrent controls used? (Concurrent preferred over historical controls.)	Yes
3.4.	If cohort study or cross-sectional study, were groups comparable on important confounding factors and/or were preexisting differences accounted for by using appropriate adjustments in statistical analysis?	N/A
3.5.	If case control or cross-sectional study, were potential confounding factors comparable for cases and controls? (If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies.)	N/A
3.6.	If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g., "gold standard")?	N/A
4.	**Was method of handling withdrawals described?**	Yes
4.1.	Were follow-up methods described and the same for all groups?	???
4.2.	Was the number, characteristics of withdrawals (i.e., dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group? (Follow up goal for a strong study is 80%.)	Yes
4.3.	Were all enrolled subjects/patients (in the original sample) accounted for?	Yes
4.4.	Were reasons for withdrawals similar across groups?	N/A
4.5.	If diagnostic test, was decision to perform reference test not dependent on results of test under study?	N/A
5.	**Was blinding used to prevent introduction of bias?**	Yes
5.1.	In intervention study, were subjects, clinicians/practitioners, and investigators blinded to treatment group, as appropriate?	???
5.2.	Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.)	Yes
5.3.	In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded?	N/A
5.4.	In case control study, was case definition explicit and case ascertainment not influenced by exposure status?	N/A
5.5.	In diagnostic study, were test results blinded to patient history and other test results?	N/A

6.	**Were intervention/therapeutic regimens/exposure factor or procedure and any comparison(s) described in detail? Were interveningfactors described?**	???
6.1.	In RCT or other intervention trial, were protocols described for all regimens studied?	Yes
6.2.	In observational study, were interventions, study settings, and clinicians/provider described?	N/A
6.3.	Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect?	???
6.4.	Was the amount of exposure and, if relevant, subject/patient compliance measured?	???
6.5.	Were co-interventions (e.g., ancillary treatments, other therapies) described?	No
6.6.	Were extra or unplanned treatments described?	No
6.7.	Was the information for 6.4, 6.5, and 6.6 assessed the same way for all groups?	???
6.8.	In diagnostic study, were details of test administration and replication sufficient?	N/A
7.	**Were outcomes clearly defined and the measurements valid and reliable?**	???
7.1.	Were primary and secondary endpoints described and relevant to the question?	???
7.2.	Were nutrition measures appropriate to question and outcomes of concern?	Yes
7.3.	Was the period of follow-up long enough for important outcome(s) to occur?	???
7.4.	Were the observations and measurements based on standard, valid, and reliable data collection instruments/tests/procedures?	Yes
7.5.	Was the measurement of effect at an appropriate level of precision?	Yes
7.6.	Were other factors accounted for (measured) that could affect outcomes?	???
7.7.	Were the measurements conducted consistently across groups?	Yes
8.	**Was the statistical analysis appropriate for the study design and type of outcome indicators?**	Yes
8.1.	Were statistical analyses adequately described and the results reported appropriately?	Yes
8.2.	Were correct statistical tests used and assumptions of test not violated?	Yes
8.3.	Were statistics reported with levels of significance and/or confidence intervals?	Yes

	8.4.	Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)?	No
	8.5.	Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g., multivariate analyses)?	Yes
	8.6.	Was clinical significance as well as statistical significance reported?	Yes
	8.7.	If negative findings, was a power calculation reported to address type 2 error?	No
9.	**Are conclusions supported by results with biases and limitations taken into consideration?**		No
	9.1.	Is there a discussion of findings?	Yes
	9.2.	Are biases and study limitations identified and discussed?	No
10.	**Is bias due to study's funding or sponsorship unlikely?**		No
	10.1.	Were sources of funding and investigators' affiliations described?	No
	10.2.	Was the study free from apparent conflict of interest?	???